The role of the melanoma gene *MC1R* in Parkinson disease and REM sleep Behavior Disorder

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Abstract

The *MC1R* gene, suggested to be involved in Parkinson disease (PD) and melanoma, was sequenced in PD patients (n=539) and controls (n=265) from New-York, and PD patients (n=551), rapid eye movement sleep behavior disorder (RBD) patients (n=351) and controls (n=956) of European ancestry. Sixty-eight *MC1R* variants were identified, including 7 common variants with frequency>0.01. None of the common variants was associated with PD or RBD in the different regression models. In a meta-analysis with fixed-effect model, the p.R160W variant was associated with an increased risk for PD (OR=1.22, 95%CI 1.02-1.47, p=0.03) but with significant heterogeneity (p=0.048). Removing one study that introduced the heterogeneity resulted in non-significant association (OR=1.11, 95%CI 0.92-1.35, p=0.27, heterogeneity p=0.57). Rare variants had similar frequencies in patients and controls (10.54% and 10.15%, respectively, p=0.75), and no cumulative effect of carrying more than one *MC1R* variant was found. The current study does not support a role for the *MC1R* p.R160W and other variants in susceptibility for PD or RBD.

Introduction

There is an unexplained yet well-validated association between Parkinson disease (PD, MIM no. 168600) and melanoma; patients with PD have an increased risk for developing melanoma, and melanoma patients have an increased risk for PD.(Liu, et al., 2011,Pan, et al., 2011) Several hypotheses were proposed in an attempt to explain the mechanism underlying this association; however, the exact causative factors are yet to be determined. One of the hypotheses is the existence of genetic pleotropy, i.e. the same genetic risk factors for both diseases.

Large genetic studies have identified various risk factors for PD, including genome wide associated loci(Do, et al., 2011,International Parkinson Disease Genomics, et al., 2011,Nalls, et al., 2014,Satake, et al., 2009,Simon-Sanchez, et al., 2009) or mutations in specific genes such as *GBA*, *LRRK2*, *SNCA*, *VPS35*, *SMPD1*, *PARK2*, *PINK1*, *PARK7* and others (reviewed in (Gan-Or, et al., 2015,Trinh and Farrer, 2013)). However, none of these genetic loci or genes can currently explain the association between PD and melanoma. Recently, it was suggested that the melanoma-related *MC1R* gene, encoding the melanocortin 1 receptor, is associated with PD,(Tell-Marti, et al., 2015b) but this association is currently controversial.(Dong, et al., 2014,Elincx-Benizri, et al., 2014,Lubbe, et al., 2015,Tell-Marti, et al., 2015a) In a Spanish cohort of PD patients and controls, the melanoma-associated *MC1R* p.R160W variant was associated with a 2-fold increased risk for PD.(Tell-Marti, et al., 2015b) However, a previous, larger case-control study that included the *MC1R* p.R160W variant did not identify this association.(Dong, et al., 2014) Moreover, in a smaller study that included patients with PD alone, PD with melanoma, and melanoma alone, neither this variant nor other *MC1R* variants were associated with PD.(Elincx-Benizri, et al., 2014) In a Chinese cohort, in which the *MC1R* p.R160W was absent, other *MC1R* variants were also not associated with an increased risk for PD.(Foo, et al., 2015) Furthermore, the *MC1R* gene was not identified in any of the large genome wide association studies

(GWAS) (Do, et al., 2011,International Parkinson Disease Genomics, et al., 2011,Nalls, et al., 2014,Satake, et al., 2009,Simon-Sanchez, et al., 2009), and no single nucleotide polymorphism (SNP) in the *MC1R* locus was associated with PD, even without correction for multiple comparisons in the PDGene database (www.pdgene.org).

In the current study, we aimed to examine the role of the *MC1R* gene in PD. Furthermore, since rapid eye movement (REM) sleep behavior disorder (RBD), a parasomnia that is a prodromal disorder for PD and other synucleinopathies, (Postuma, 2014,Postuma, et al., 2015) the association between *MC1R* variants and RBD was also studied.

Methods

Study populations

Three populations were included in the current study: 1) a cohort of unrelated, consecutively recruited PD patients (n=539) and controls (n=265) from Columbia University, New-York, NY, USA (more details on the recruitment of cohort 1, termed "SPOT" cohort, were previously published(Alcalay, et al., 2015)). 2) a cohort of unrelated, consecutively recruited PD patients (n=551) and controls (n=956) of European ancestry, mainly French-Canadian and French, and 3) a cohort of unrelated, consecutively recruited RBD patients (n=351) of European ancestry, also mainly French-Canadian and French. Cohort 2 was collected through a network of collaborators from France and Quebec, including the Quebec Parkinson's Network (http://rpq-qpn.ca/). Cohort 3 was collected at the Montreal Neurological Institute (MNI), Montreal, Canada, by collaborators from the International RBD Study Group (Postuma, et al., 2015,Schenck, et al., 2013) from Europe and Canada. Basic demographic characteristics of these cohorts are detailed in Table 1. The control population from the MNI was composed of ethnicity-matched elderly controls (n=553, average age 51.8 ± 13.2 years) and young controls (n=403, average age 31.9 ± 4.9 years). Since the frequencies of the tested variants were nearly identical in these two control populations (see results), they could be reliably combined for the analysis, after adjusting for the age differences in the association analyses (see methods). Lack of relatedness and the ancestry in cohorts 2 and 3 were ascertained by unpublished genome-wide association study data, and in cohort 1 it was ascertained by the clinician who recruited and routinely treating the patients. PD patients were diagnosed according to the UK Brain Bank Criteria (but without excluding patients who reported family history of PD) by neurologists specialized in movement disorders. RBD was diagnosed according to the International Classification of Sleep Disorders criteria (ICSD-2) by neurologists specialized in sleep disorders, based on both history and polysomnography showing REM sleep without atonia. All patients and controls

signed an informed consent form before entering the study, and the protocols were approved by the respective institutional review boards.

Sequencing of the *MC1R* gene

DNA was extracted by using a standard salting-out protocol. The entire coding sequence of the *MC1R* gene (NM_002386) was amplified by using the forward primer 5' GCAGCACCATGAACTAAGCA 3' and the reverse primer 5' CAGGGTCACACAGGAACCA 3' with the AmpliTaq Gold DNA Polymerase (Applied Biosystems, CA, USA) or the *Taq* DNA polymerase (Qiagen, Maryland, USA). The amplified products were then sequenced using the forward primer 5' AACCTGCACTCACCCATGTA 3' and the reverse primer 5' TTTAAGGCCAAAGCCCTGGT 3' at the Genome Quebec Innovation Centre (Montréal, QC, Canada) using a 3730XL DNAnalyzer (Applied Biosystems, CA, USA). The sequences were analyzed using the Genalys 3.3b software. All variants that were identified in the forward sequencing were also identified in the reverse sequencing in the overlapping region. Furthermore, forward and reverse sequencing of 40 samples was repeated from another tube of DNA that was taken in another visit of the patient, with a 100% match. Only samples with both forward and reverse sequencing were included in the analysis.

Statistical analysis

To compare single categorical variables, χ^2 or Fisher's exact test was used, and to compare continuous variables, Student's t-test or ANOVA was used. χ^2 with one degree of freedom was used to determine whether the observed genotype frequencies of the common *MC1R* variants deviate from the expected frequencies based on Hardy-Weinberg Equilibrium (HWE). To estimate the association between the detected common *MC1R* variants and PD or RBD, binary logistic regression with the status of the individual (patient or control) as the dependent variable was used. When patients and controls did not

match for sex or age, these variables were added as covariates for the analysis to adjust for their effects. When a regression model for all patients from both centers (NY and Montreal) was performed, the site was also added as covariate to adjust for the differences in the genetic background of the two populations from the two centers. Power analysis demonstrated that our population had a power of >98% to detect the originally reported association between the *MC1R* p.R160W and PD (Tell-Marti, et al., 2015b). Furthermore, our PD population (1090 patients and 1221 controls) had a power of >80% to detect a much lower odds ratio of 1.4. All the statistical analysis, except for the meta-analysis, was performed using the SPSS v.21 software (IBM, Ltd.). The meta-analysis was performed by using an R package (Metafor). Data for the meta-analysis were collected and weighted at the individual level, and heterozygous and homozygous carriers were considered as carriers for the analysis. The Cochran-Mantel-Haenszel test was used to pool the studies and calculate the odds ratios (OR) using either the fixed- or random-effect models. Tarone's test was applied to estimate heterogeneity, and in case of significant heterogeneity, the source of heterogeneity was identified by excluding studies one by one, and re-calculating the Tarone's test for heterogeneity. The online tools SIFT(Kumar, et al., 2009) and PolyPhen-2 (Adzhubei, et al., 2010) were used to predict the effects of the *MC1R* variants.

Results

Common and rare *MC1R* variants have no or minimal association with the risk for PD or RBD

A total of 68 *MC1R* variants were identified in patients and controls. Supplementary Table 1 details these variants, their predicted effect on the protein, and their distribution among patients and controls. Seven common variants with allele frequency > 0.01 were included in logistic regression models to determine their association with the risk for PD and RBD (Table 2). None of these variants deviated from Hardy-Weinberg equilibrium (HWE). First, PD patients and RBD patients were separately compared to their respective control populations from each center. Subsequently, a combined analysis of the PD and RBD patients from Montreal vs. their controls, and a combined analysis of all PD and RBD patients and controls from both centers were performed. The elderly and young controls from Montreal had nearly identical frequencies for the seven common variants (0.29 and 0.30 for the p.V60L variant, respectively, 0.13 and 0.13 for p.V92M, 0.11 and 0.10 for p.R151C, 0.08 and 0.08 for p.R160W, 0.07 and 0.08 for p.R163Q, 0.04 and 0.04 for p.D294H, and 0.17 and 0.17 for p.T314T) and could therefore be combined reliably as one control group. None of the seven common *MC1R* variants was associated with risk for PD or RBD in any of the models (Table 2). The *MC1R* p.R160W, which was suggested to be associated with PD,(Tell-Marti, et al., 2015b) had non-significant odd ratios (ORs) ranging between 0.76-1.13 in the different analyses (uncorrected *p* value > 0.45 in all the analyses, Table 2). Of the seven common variants, six were nonsynonymous, and similar analysis of these six variants alone resulted in very similar, non-significant results (data not shown).

The combined frequency of all the rare variants (allele frequency < 0.01) was nearly identical among patients and controls (10.54% and 10.15%, respectively, p=0.75, Fischer exact test). The combined frequency of rare nonsynonymous, frameshift or stop mutations was also very similar among patients and controls (8.53% and 8.68%, respectively, p=0.44, Fisher's exact test). Furthermore, no

cumulative effect of carrying more than one *MC1R* variant was found (Figure 1). Several rare variants had higher frequencies in patients compared to controls (Supplementary Table 1), but none of these variants reached statistical significance after correction for multiple comparisons.

Meta-analysis of the effect of the *MC1R* p.R160W variant on PD risk demonstrates minimal or no effect.

In order to further determine whether the *MC1R* p.R160W is associated with the risk for PD as was previously suggested,(Tell-Marti, et al., 2015b) a meta-analysis of four populations where this variant was specifically analyzed (two that were previously published and two from the current study, Table 3) was performed. Both fixed- and random-effect models were used (Figure 2A and 2B, respectively). In the fixed-effect model, the *MC1R* p.R160W was associated with an increased risk for PD (OR = 1.22, 95% CI 1.02-1.47, p=0.03, Figure 2A). However, there was significant heterogeneity in this model (p=0.048), which was introduced by the only study that had previously demonstrated an association between this variant and PD,(Tell-Marti, et al., 2015b) rendering the meta-analysis results less reliable. After the exclusion of this study, there was no significant association (OR = 1.11, 95% CI 0.92-1.35, p=0.27), and the heterogeneity was not significant (p=0.57, Figure 2C). In the random-effect model, the *MC1R* p.R160W was not associated with an increased risk for PD (OR = 1.29, 95% CI 0.90-1.85, p=0.17, p for heterogeneity = 0.048, Figure 2B). In this model too, the exclusion of the same study resulted in non-significant association with non-significant heterogeneity (Figure 2D). When other studies rather than the original study describing this suggested association are being removed from the meta-analysis, the heterogeneity remains significant, demonstrating that this study (Tell-Marti, et al., 2015b) is the source of heterogeneity.

Discussion

The current study demonstrates that variants in the *MC1R* gene have minimal or no association with the risk for PD or RBD. More specifically, the *MC1R* p.R160W variant, which was suggested to be a risk factor for PD,(Tell-Marti, et al., 2015b) was not associated with PD or RBD when comparing specific populations or in the meta-analysis. The discrepancies between the single study that suggested an association between the *MC1R* p.R160W variant and the current study could be explained by the different populations used. It is possible, for example, that in different populations, other genetic or environmental factors exist that differentially modify the association of *MC1R* with PD. However, if this variant is hypothesized to be the functional variant that increases the risk for PD, by affecting the function of the melanocortin 1 receptor, it should have relatively similar effects in each population. Further support for the lack of association of *MC1R* with PD is provided by the meta-OR previously reported for this variant (OR=0.98, 95% CI 0.89-1.07, p=0.62) in the International PD Genomic Consortium data,(Dong, et al., 2014) and the lack of signal in this locus in the PDGene database (www.pdgene.org). Although we cannot decisively rule out that this variant has a minor role in PD susceptibility that can only be detected in a much larger meta-analysis, the current data suggest that the *MC1R* gene, and specifically the p.R160W variant, are probably not associated with PD, or have a very small effect. A previous observation from a cohort of 272 PD patients and 1185 controls, which suggested that the p.R151C variant is associated with PD,(Gao, et al., 2009) was also not supported by our results.

From a purely genetic perspective, there is not enough evidence that currently points to a specific shared genetic background; however, a few interesting observations were made. The largest GWASs from both diseases showed no overlap between the associated loci,(Law, et al., 2015,Nalls, et al., 2014) and a study that specifically targeted known PD loci in a large melanoma cohort failed to identify an association.(Meng, et al., 2012) However, one of the GWAS loci that was identified in melanoma(Meng,

et al., 2012) and melanocytic cutaneous nevi(Falchi, et al., 2009) cohorts is the *PLA2G6* locus. Interestingly, mutations in *PLA2G6* may cause PD or Parkinsonism-dystonia syndrome,(Gui, et al., 2013,Kauther, et al., 2011,Malaguti, et al., 2015,Paisan-Ruiz, et al., 2009) suggesting a potential genetic link between the two conditions.(Paisan-Ruiz and Houlden, 2010) Another intriguing locus that was identified in PD GWAS is around the *GPNMB* gene,(Nalls, et al., 2014) which codes for the glycoprotein non-metastatic melanoma protein B, which may have an important role in melanoma.(Maric, et al., 2013,Tomihari, et al., 2010) Additional studies are needed to determine if these genes may contribute to the co-occurrence of PD and melanoma. In the current study, data on melanoma occurrence in the PD and RBD cohorts were not available.

In order to determine whether genetics, environmental factors, or their interaction leads to the co-occurrence of PD and melanoma, large genetic-environmental studies are needed. To reach statistical power that will allow drawing strong conclusions, large collaborations between different centers are needed to reach the number of patients required for such analysis. Understanding the underlying causes of this co-morbidity is of great importance, since it may allow specific interventions in PD patients or in prodromal PD to prevent melanoma.

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Conflict of interest statement

The authors report no conflict of interests.

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Legends to figures

Figure 1.

Cumulative carriage of *MC1R* variants in the different study populations.

A. The carriage frequencies of one or more *MC1R* variants were similar among PD patients, RBD patients and controls collected at the MNI ($p>0.05$ for all comparisons) **B.** The carriage frequencies of one or more *MC1R* variants were similar among PD patients and controls collected at Columbia, NY ($p>0.05$ for all comparisons).

Figure 2.

Meta-analyses of the *MC1R* p.R160W variant.

A. Meta-analysis of the *MC1R* p.R160W in four studies under the fixed-effect model. While three studies had non-significant ORs of 0.91 – 1.28, one study alone (Tell-Marti et al.) drove the association. However, these results cannot be considered statistically significant due to the significant heterogeneity ($p=0.048$). **B.** Meta-analysis of the *MC1R* p.R160W in four studies under the random-effect model. Here too, one study introduced a significant heterogeneity to the model (Heterogeneity $p=0.048$) **C.** Meta-analysis of the *MC1R* p.R160W in three studies, excluding the one study that introduced heterogeneity, under the fixed-effect model (OR=1.11, 95% CI 0.92-1.35, $p=0.27$, heterogeneity $p=0.57$). **D.** Meta-analysis of the *MC1R* p.R160W in three studies, excluding the one study that introduced heterogeneity, under the random-effect model, resulting in identical OR estimates and statistics as the fixed-effect model.

Table 1. Demographic characteristics of the study populations

	MNI[a]				New York	
	PD	**RBD**	**Total PD + RBD**	**Controls**	**PD**	**Controls**
Number	551	351	902	956	539	265
Men, n (%) [b]	346 (63.7%)	264 (78.6%)	610 (69.4%)	480 (50.3%)	346 (64.2%)	92 (34.7%)
Age (± SD) [c]	65.7 (± 9.7)	67.5 (± 8.7)	66.4 (± 9.4)	43.5 (± 14.4)	65.9 (± 10.6)	64.8 (± 10.4)

PD, Parkinson disease; RBD, rapid eye movement sleep behavior disorder; SD, standard deviation

[a] MNI, Montreal Neurological Institute, Montreal, Canada, is the center where samples of European ancestry where collected through international collaborations as detailed in the methods.

[b] Data on gender was not available for 8 PD, 15 RBD and one control from Montreal.

[c] Data on age was not available for 14 PD, 41 RBD, 10 control from Montreal, and for one PD and 3 controls from Columbia, NY.

Table 2. Association of common *MC1R* variants with risk for PD or RBD

	Minor allele frequency					OR (95% CI), *p* value [a]				
Variant	**PD MNI (n=551)**	**RBD MNI (n=351)**	**Control MNI (n=956)**	**PD NY (n=539)**	**Control NY (n=265)**	**A [b]**	**B [c]**	**C [d]**	**D [e]**	**E [f]**
p.V60L	0.142	0.121	0.156	0.195	0.200	0.83 (0.60-1.15), *p*=0.26	0.72 (0.47-1.09), *p*=0.12	0.80 (0.59-1.09), *p*=0.15	0.91 (0.65-1.28), *p*=0.61	0.83 (0.67-1.03), *p*=0.10
p.V92M	0.066	0.068	0.067	0.070	0.072	1.01 (0.47-2.15), *p*=0.99	1.15 (0.47-2.80), *p*=0.77	1.03 (0.52-2.05), *p*=0.93	0.90 (0.45-1.78), *p*=0.75	0.88 (0.55-1.41), *p*=0.60
p.R151C	0.054	0.064	0.054	0.065	0.070	0.92 (0.57-1.47), *p*=0.72	1.14 (0.67-1.96), *p*=0.63	1.05 (0.69-1.60), *p*=0.81	0.77 (0.48-1.23), *p*=0.27	0.90 (0.67-1.23), *p*=0.52
p.R160W	0.048	0.037	0.039	0.045	0.053	1.13 (0.68-1.90), *p*=0.63	0.76 (0.38-1.54), *p*=0.45	1.10 (0.67-1.80), *p*=0.72	0.92 (0.53-1.57), *p*=0.76	0.96 (0.68-1.35), *p*=0.80
p.R163Q	0.024	0.036	0.036	0.064	0.047	0.80 (0.43-1.50), *p*=0.49	1.26 (0.61-2.63), *p*=0.53	0.92 (0.52-1.62), *p*=0.77	1.30 (0.77-2.21), *p*=0.33	1.09 (0.76-1.58), *p*=0.63
p.D294H	0.018	0.014	0.021	0.011	0.019	0.64 (0.32-1.29), *p*=0.21	0.46 (0.18-1.21), *p*=0.12	0.60 (0.31-1.17), *p*=0.13	0.68 (0.28-1.68), *p*=0.41	0.67 (0.40-1.11), *p*=0.12
p.T314T	0.085	0.093	0.091	0.102	0.102	0.92 (0.46-1.82), *p*=0.80	0.83 (0.37-1.88), *p*=0.65	0.90 (0.48-1.67), *p*=0.73	0.99 (0.54-1.81), *p*=0.98	1.03 (0.68-1.56), *p*=0.90

OR, odds ratio; CI, confidence interval, PD MNI, Parkinson disease samples collected at the Montreal Neurological Institute; RBD MNI, REM sleep Behavior Disorder patients collected at the Montreal Neurological Institute, through the International RBD study group; PD NY, Parkinson disease patients collected at Columbia University, New-York; Control MNI, controls collected at the Montreal Neurological Institute; Control NY, controls collected at Columbia University, New-York.

Variants were called according to NM_002386.
[a] Bonferroni correction for multiple comparisons set the cut-of *p* value for statistical significance at $p<0.0071$.
[b] *p* value comparing PD MNI to Control MNI using a regression model, adjusted for gender and age
[c] *p* value comparing RBD MNI to Control MNI using a regression model, adjusted for gender and age
[d] *p* value comparing PD MNI + RBD MNI to Control MNI using a regression model, adjusted for gender and age
[e] *p* value comparing PD NY to Control NY using a regression model, adjusted for gender and age
[f] *p* value comparing all patients (PD MNI + RBD MNI + PD NY) to all controls (Control MNI + Control NY) using a regression model, adjusted for site, gender and age

Table 3.

Populations included in meta-analysis of the *MC1R* p.R160W variant in PD.

Study	**population**	**Number of PD patients, (% carriers of the *MC1R* p.R160W variant)[a]**	**Number of controls (% carriers of the *MC1R* p.R160W variant)**
Dong et al.[13]	Non-Hispanic whites	777 (13.6%)	1550 (12.5%)
Tell-Marti et al.[11]	Caucasians from Spain	870 (5.0%)	736 (2.0%)
Current study	Mainly French-Canadian / French	551 (9.6%)	956 (7.8%)
Current study	North-American from NY	539 (8.9%)	265 (9.8%)
Current study total		1090 (9.3%)	1221 (8.2%)

[a] Since RBD patients may convert to other synucleinopathies, such as Dementia with Lewy Bodies and Multiple System Atrophy, they were not included in the meta-analysis for the effect of the *MC1R* p.R160W variant on PD risk.

Figure 1

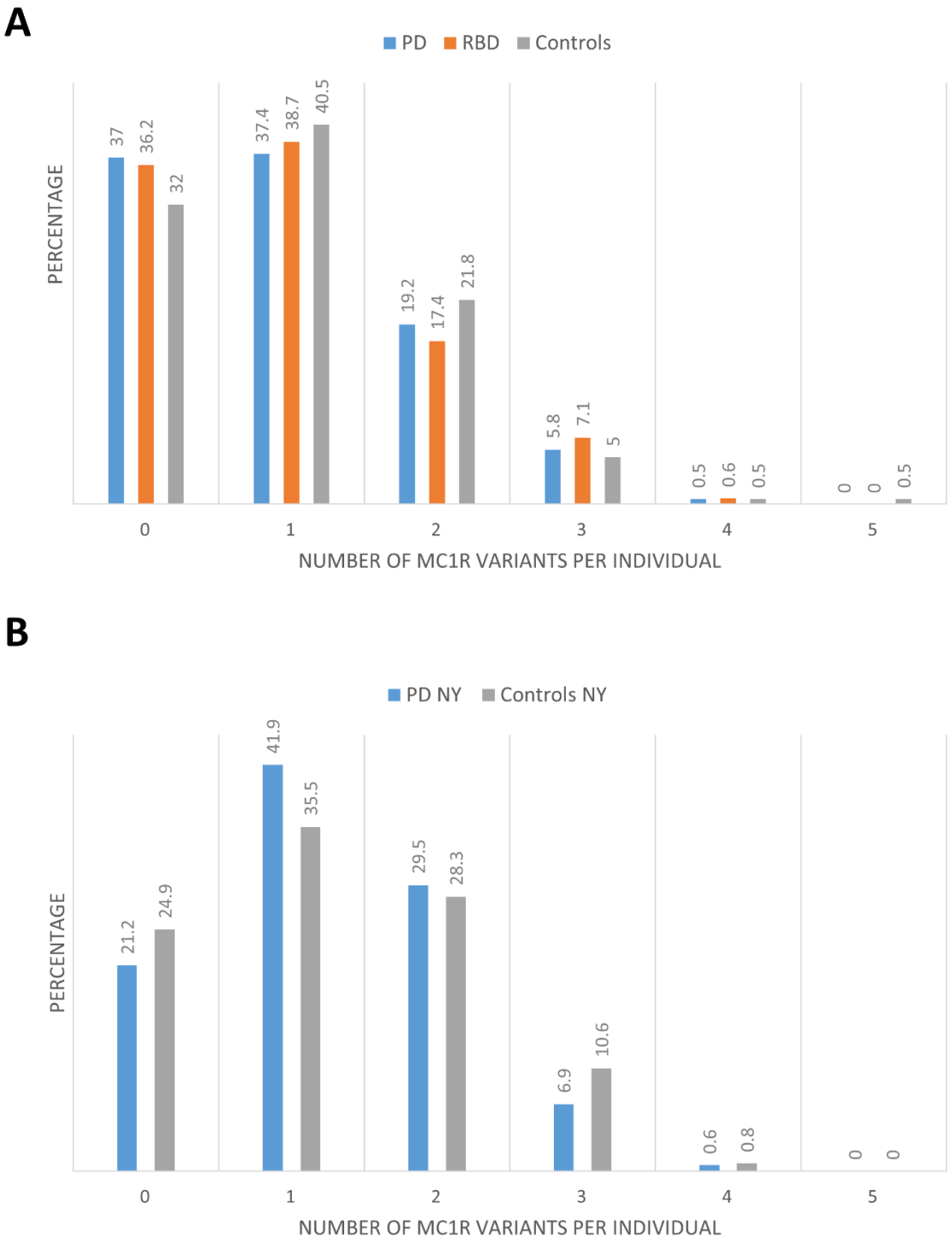
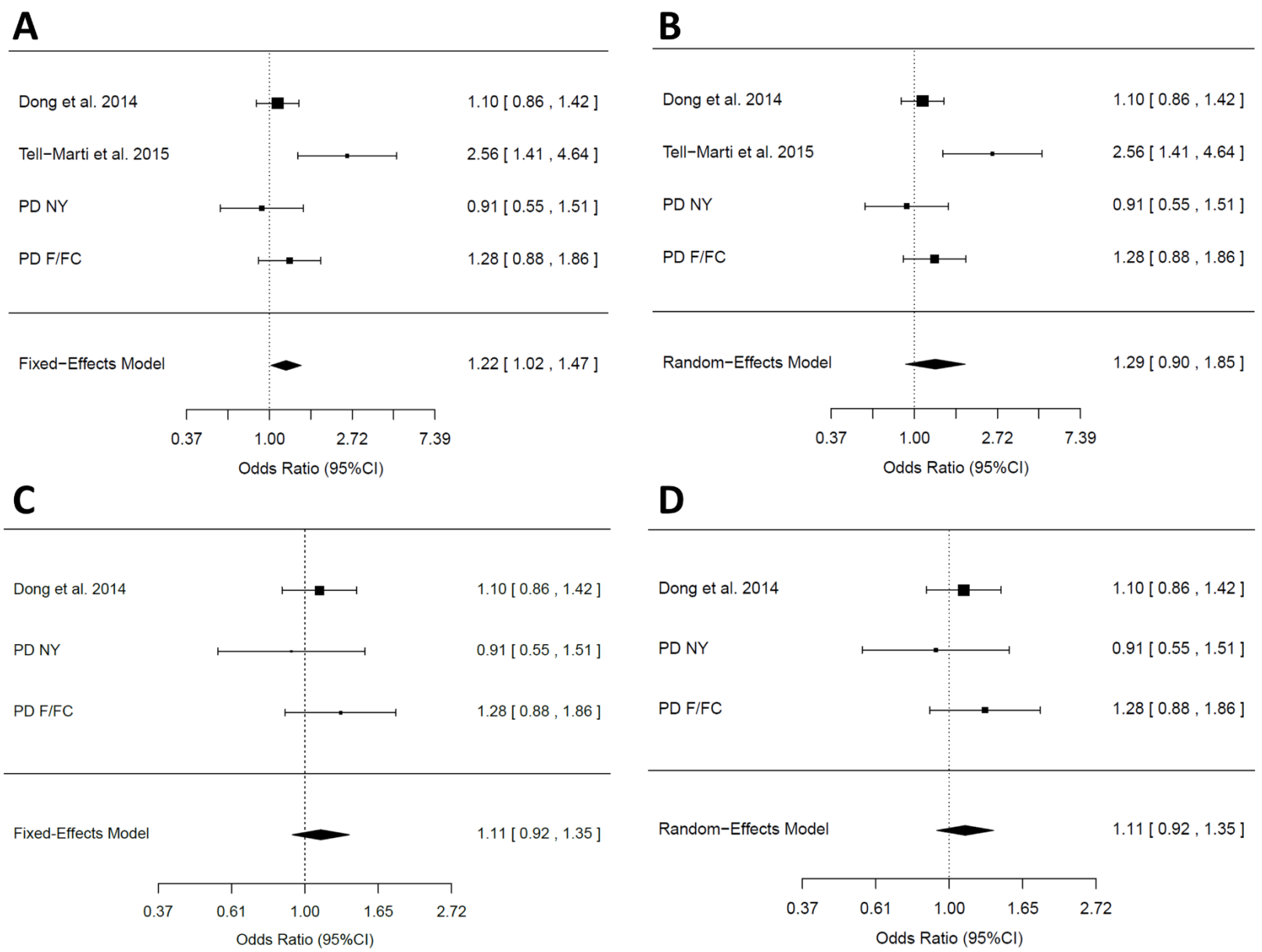

A
PD
RBD
Controls
PERCENTAGE
37
36.2
32
37.4
38.7
40.5
19.2
17.4
21.8
5.8
7.1
5
0.5
0.6
0.5
0
0
0.5
0
1
2
3
4
5
NUMBER OF MC1R VARIANTS PER INDIVIDUAL
B
PD NY
Controls NY
PERCENTAGE
21.2
24.9
41.9
35.5
29.5
28.3
6.9
10.6
0.6
0.8
0
0
0
1
2
3
4
5
NUMBER OF MC1R VARIANTS PER INDIVIDUAL

Figure 2


A
Dong et al. 2014
1.10 [0.86 , 1.42]
Tell−Marti et al. 2015
2.56 [1.41 , 4.64]
PD NY
0.91 [0.55 , 1.51]
PD F/FC
1.28 [0.88 , 1.86]
Fixed−Effects Model
1.22 [1.02 , 1.47]
0.37
1.00
2.72
7.39
Odds Ratio (95%CI)
B
Dong et al. 2014
1.10 [0.86 , 1.42]
Tell−Marti et al. 2015
2.56 [1.41 , 4.64]
PD NY
0.91 [0.55 , 1.51]
PD F/FC
1.28 [0.88 , 1.86]
Random−Effects Model
1.29 [0.90 , 1.85]
0.37
1.00
2.72
7.39
Odds Ratio (95%CI)
C
Dong et al. 2014
1.10 [0.86 , 1.42]
PD NY
0.91 [0.55 , 1.51]
PD F/FC
1.28 [0.88 , 1.86]
Fixed-Effects Model
1.11 [0.92 , 1.35]
0.37
0.61
1.00
1.65
2.72
Odds Ratio (95%CI)
D
Dong et al. 2014
1.10 [0.86 , 1.42]
PD NY
0.91 [0.55 , 1.51]
PD F/FC
1.28 [0.88 , 1.86]
Random−Effects Model
1.11 [0.92 , 1.35]
0.37
0.61
1.00
1.65
2.72
Odds Ratio (95%CI)

Supplementary Table 1

***MC1R* variants identified in the current study.**

ID	DNA variant	Protein Variant	SIFT score	PolyPhen2 score	Genotype distribution in patients (n=1441) [a]	Genotype distribution in controls (n=1221) [a]
-	c.52C>G	p.P18A	-	-	0/0/1441	0/1/1220
rs312262906	fs c.84insA		-	-	0/4/1437	0/4/1217
rs369016553	c.101G>A	p.R34Q	0.68	0	0/1/1440	0/0/1221
rs376679503	c.100C>T	p.R34W	0.15	0	0/1/1440	0/0/1221
rs61996344	c.133T>C	p.F45L	0	0.999	0/2/1439	0/1/1220
-	c.140G>C	p.S47T	-	-	0/0/1441	0/1/1220
rs555179612	fs c.536insC		-	-	0/0/1441	0/3/1218
rs117952179	c.170C>T	p.A57V	0.63	0.001	0/2/1439	0/0/1221
rs1805005	c.178G>T	p.V60L	0.34	0.98	45/361/1035	32/341/848
rs34090186	c.200G>A	p.R67Q	0.03	0.248	0/1/1440	0/0/1221
rs527493051	c.243C>A	p.A81A	-	-	0/1/1440	0/0/1221
-	c.241G>C	p.A81P	-	-	0/0/1441	0/1/1220
rs34474212	c.247T>C	p.S83P	0	0.988	0/6/1435	0/4/1217
rs1805006	c.252C>A	p.D84E	0	0.936	0/25/1416	0/23/1198
rs2228479	c.274G>A	p.V92M	0.1	0.047	9/178/1254	6/154/1061
rs34158934	c.284C>T	p.T95M	0.06	0.723	0/3/1438	0/3/1218
rs573416648	c.285G>A	p.T95T	-	-	0/2/1439	0/1/1220
rs373341896	c.292A>G	p.I98V	0.08	0.007	0/0/1441	0/1/1220
-	c.295C>A	p.L99T	-	-	0/0/1441	0/1/1220
rs2229617	c.310G>A	p.G104S	0.04	0.327	0/0/1441	0/2/1219
rs3212364	c.318G>A	p.L106L	-	-	0/1/1440	0/0/1221
-	c.326G>A	p.R109Q	-	-	0/0/1441	0/1/1220
-	c.329C>G	p.A110G	-	-	0/1/1440	0/0/1221
rs33932559	c.359T>C	p.I120T	0.03	0.033	0/1/1440	0/1/1220
rs201192930	c.364G>A	p.V122M	0.15	0.305	0/4/1437	0/1/1220
rs372353477	c.366G>A	p.V122V	-	-	0/1/1440	0/0/1221
rs370094672	c.392G>A	p.S131N	0	0.979	0/1/1440	0/3/1218
rs201429598	c.399C>T	p.C133C	-	-	0/1/1440	0/2/1219
rs372929572	c.417C>G	p.A139A	-	-	0/1/1440	0/0/1221
rs11547464	c.425G>A	p.R142H	0	0.992	0/19/1422	0/15/1206
rs374423188	c.445G>A	p.A149T	0.03	0.678	0/1/1440	0/0/1221
rs1805007	c.451C>T	p.R151C	0.02	0.982	7/160/1274	4/133/1084

rs201827012	c.453C>G	p.R151R	-	-	0/1/1440	0/2/1219
rs201326893	c.456C>A	p.Y152*	-	-	0/9/1432	0/3/1218
-	c.459C>T	p.H153H	-	-	0/1/1440	0/0/1221
rs1110400	c.464T>C	p.I155T	0	0.864	0/24/1417	0/24/1197
rs201975178	c.467T>C	p.V156A	0.01	0.816	0/0/1441	0/1/1220
rs1805008	c.478C>T	p.R160W	0	0.672	2/124/1315	2/98/1121
-	c.479G>A	p.R160Q	-	-	0/2/1439	0/0/1221
rs885479	c.488G>A	p.R163Q	0.3	0.032	10/100/1331	2/92/1127
rs367985661	c.492C>T	p.A164A	-	-	0/1/1440	0/0/1221
rs35040147	c.497C>G	p.A166G	0.21	0.169	0/1/1440	0/0/1221
rs376670171	c.515G>T	p.S172I	0	0.949	0/1/1440	0/0/1221
rs370040645	c.546C>T	p.Y182Y	-	-	0/0/1441	0/1/1220
COSM1269522	c.549C>T	p.Y183Y	-	-	0/0/1441	0/1/1220
-	c.554A>G	p.H185R	-	-	0/0/1441	0/1/1220
COSM3969769	c.577G>A	p.V193M	0	0.532	0/0/1441	0/1/1220
rs374355873	c.607A>G	p.M203V	0.03	0.868	0/0/1441	0/1/1220
-	c.622G>C	p.V208L	-	-	0/1/1440	0/0/1221
COSM4063685	c.637C>T	p.R213W	0	0.079	0/1/1440	0/3/1218
-	c. fs642-655del		-	-	0/1/1440	0/0/1221
rs372152373	c.667C>G	p.R223G	0.08	0.417	0/0/1441	0/1/1220
rs372152373	c.667C>T	p.R223W	0	0.047	0/0/1441	0/2/1219
COSM3421247	c.678G>A	p.K226K	-	-	0/3/1438	0/0/1221
rs146544450	c.699G>A	p.Q233Q	-	-	0/10/1431	0/8/1213
rs369402699	c.729C>T	p.L243L	-	-	0/0/1441	0/1/1220
rs200215218	c.766C>T	p.P256S	0	1	0/5/1436	0/0/1221
COSM3421249	c.789C>A	p.L263L	-	-	0/1/1440	0/0/1221
rs181269865	c.792C>T	p.I264I	-	-	0/1/1440	0/3/1218
rs201171524	c.832A>G	p.K278E	0.02	0.595	0/1/1440	0/0/1221
rs202197434	c.837C>A	p.N279K	0.05	0.911	0/2/1439	0/0/1221
-	c.861C>A	p.I287I	-	-	0/1/1440	0/0/1221
rs1805009	c.880G>C	p.D294H	0	0.996	0/42/1399	0/51/1170
rs370472871	c.895G>A	p.A299T	0.01	0.465	0/1/1440	0/0/1221
rs3212367	c.900C>T	p.F300F	-	-	0/0/1441	0/1/1220
rs2228478	c.942A>G	p.T314T	-	-	16/237/1188	15/198/1008
rs377248188	c.944G>C	p.C315S	0	0.06	0/1/1440	0/0/1221
rs151318945	c.948C>T	p.S316S	-	-	0/4/1437	0/1/1220

Variants are called according to NM_002386

[a] SIFT score lower that 0.05 is considered to predict a deleterious variant.

[b] PolyPhen scores of 0.15-0.85 may be considered as possibly damaging, and >0.85 as probably damaging

[c] The genotype distribution represents the number of carriers of homozygous / heterozygous / non-carriers of each variant.